FLOWERING PHENOLOGY AND FLORAL VISITORS IN DISTYLOUS POPULATIONS OF *PSYCHOTRIA CARTHAGENENSIS* (RUBIACEAE) IN BRAZILIAN CERRADO[1]

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ABSTRACT

Flowering synchrony has a relevant role in pollinator attraction. In distylous species, synchronous blooming between individuals of both morphs means that the chances of intermorph crosses are increased, which is important for the maintenance of the heterostylous system. The aim of the present study was to describe the flowering phenology and the behavior of the floral visitors in three populations of *Psychotria carthagenensis* Jacq. (Rubiaceae) between January 2008 and January 2010. Flower and pollinator censuses were conducted at three urban forest fragments in Brazil. *Psychotria carthagenensis* flowering occurred between October and December, with a high degree of synchrony within populations as well as between floral morphs. The exotic *Apis mellifera* L. (Apidae) was the main pollinator in the three populations. A high degree of similarity was found in the behavior of floral visitors as well as in nectar production. Visitation rates did not differ between floral morphs. Similar floral recompenses (flower availability and nectar) as well as no preference in pollinators' visiting are thought to be contributing to a greater intermorph visitation to pin and thrum flowers in *P. carthagenensis*.

Key words: Distyly, floral polymorphism, flowering synchrony, heterostyly, pollination.

Heterostyly is a genetic polymorphism characterized by the occurrence of two (distyly) or three (tristyly) floral morphs in a given population (Barrett, 1992a). Heterostylous populations could present an equal balance in morph ratio (isoplethy) as well as deviation of morph frequencies in a 1:1 ratio (anisoplethy). Within the Rubiaceae, distyly is mainly documented in the Rubioideae and is characteristic of the Psychotrieae (Barrett & Richards, 1990). In typically distylous species among the angiosperms, pin flowers have long styles and short stamens, whereas thrum flowers have short styles and long stamens (Ganders, 1979).

Beyond the reciprocal positioning of the anthers and stigmas and heteromorphic incompatibility, ancillary polymorphisms occur in typical distylous systems, such as morph-specific differences in corolla length, morphology and quantity of pollen grains, and size and morphology of the stigma and stigmatic papillae (Vuilleumier, 1967; Ganders, 1979; Barrett & Richards, 1990; Hamilton, 1990; Castro et al., 2004). These differences between floral morphs can lead to differences in the most effective pollen vectors (Lau & Bosque, 2003), which, in turn, can result in loss or modification of heteromorphic incompatibility (see Pérez-Alquicira et al., 2010; Sosenski et al., 2010). Variations in floral morphology, incompatibility systems, and morph ratios between populations are well-documented for distylous species (e.g., Sobrevilla et al., 1983; Li & Johnston, 2001; Sakai & Wright, 2008; Ferrero et al., 2011a, 2011b).

Heterostyly traditionally has been seen as a mechanism that promotes cross-pollination and reduces pollen waste (Darwin, 1877; Lloyd & Webb, 1992a, 1992b), thereby suggesting disassortative pollination, in which there is greater pollen flow to the opposing morph (Darwin, 1877; Charlesworth &

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Charlesworth, 1979; Lloyd & Webb, 1992b; Barrett, 2002). If pollen flow is disassortative and both morphs have equal functional potential, both are expected to exhibit equivalent male and female reproductive success (García-Robledo, 2008). In distylous species, asymmetrical pollen flow between morphs is common (Lau & Bosque, 2003). Deviations in the pollen flow pattern in heterostylous species have generally been associated with the absence of specialized pollinators (Ornduff, 1971; Björkman, 1995) as well as alterations in the foraging behavior of pollinators (Wolfe & Barrett, 1989). Also, variations in floral display may account for differences between morphs regarding pollen deposition rate and female reproductive success (Brys & Jacquemyn, 2010), due to the effects of floral display on the attraction and visitation rates of pollinators. Therefore, understanding how plant-pollinator interactions influence reproductive success is essential for understanding the factors that affect the maintenance and evolution of heterostyly (Thompson & Dommée, 1993; Stone, 1996).

The periodicity of flowering is critical to reproduction for the plants (Rathcke & Lacey, 1985) and is regulated by endogenous characteristics related to biotic and abiotic factors that are selective to flowering development (Van Schaik et al., 1993). Additionally, there is a consensus that floral display affects the fitness of the plants through pollination processes (Van Schaik et al., 1993; Fenner, 1998; Sakai, 2001). At the population level, for instance, some degree of flowering synchrony is needed so that floral display and pollinator attraction are enhanced, thereby increasing the chance of crossing between individuals (Rathcke & Lacey, 1985). In distylous species, flowering synchrony between individuals of both morphs means that the chances of intermorph crosses are increased, which is integral to the maintenance of the heterostylous system (Ganders, 1979). The effectiveness of pollination depends also on the attractiveness of floral resources offered, nectar being the resource most often presented by distylous species (Ganders, 1979). Differences in nectar composition and volume in distylous species are unusual (Ree, 1997; Legee & Wolfe, 2002; Lau & Bosque, 2003), but when they occur it is possibly due to phenotypic selection. In this instance, the pollinators respond by visiting more frequently flowers of the morph that produces the more abundant nectar (Ornelas et al., 2004; Cawoy et al., 2006). Resulting differences in visiting frequencies between floral morphs could have consequences for the maintenance of isoplethic ratios in these populations (Wolfe & Barrett, 1987).

The distylous species *Psychotria carthagenensis* Jacq. is a shrub measuring 2–3 m in height that occurs from the United States to Argentina (Hamilton, 1990). In Brazil, previous studies addressing the floral morphology and reproductive system in this species have recorded considerable variation in the reproductive characteristics, such as a case of homostyly in the northeastern region of the country (Demetrio & Machado, 2005), self-incompatibility in an anisoplethic population (Pereira, 2007) and in isoplethic populations (Koch et al., 2010; Faria et al., 2012) in the central-western region, and an instance of self-compatibility in a monomorphic population in the southeastern region (Consolaro et al., 2011). As a distylous species, morphs in populations of *P. carthagenensis* are expected to exhibit flowering synchrony to allow intermorph crosses. This can be facilitated further by similar flower availability, allowing floral visitors an equal opportunity to encounter both morphs. Despite the importance of these aspects, they remain unexplored in studies of distyly (see Ganders, 1979; Barrett & Richards, 1990; Barrett, 1992b, 2002), with no reports on pollinator performance in subsequent years and different populations. Thus, the present study seeks to answer the following questions in three populations that are both isoplethic and self-compatible (Faria et al., 2012): (1) what is the flowering period and the degree of flowering synchrony among *P. carthagenensis* individuals in each population?; (2) what is the degree of flowering synchrony between the two floral morphs of *P. carthagenensis* in terms of periodicity and floral display size?; (3) are there differences in nectar production between the two floral morphs of *P. carthagenensis*?; and (4) what is the degree of similarity in the pollinators' array and visitation rates between the floral morphs of *P. carthagenensis*?

Materials and Methods

STUDY AREA

The three populations studied for *Psychotria carthagenensis* occur in savanna fragments in protected areas located within an urban matrix in the municipality of Campo Grande (approximately 700 m.s.m.), in the state of Mato Grosso do Sul, Brazil. These areas were located in the Prosa State Park (PSP, 135 ha, 20°27′00″S, 54°33′46″W), in the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS, 35 ha, 20°29′58″S, 54°36′50″W), and in the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, 175 ha, 20°25′41″S, 54°43′03″W), which are separated from each other by a mean distance of 12.45 km. Plants of

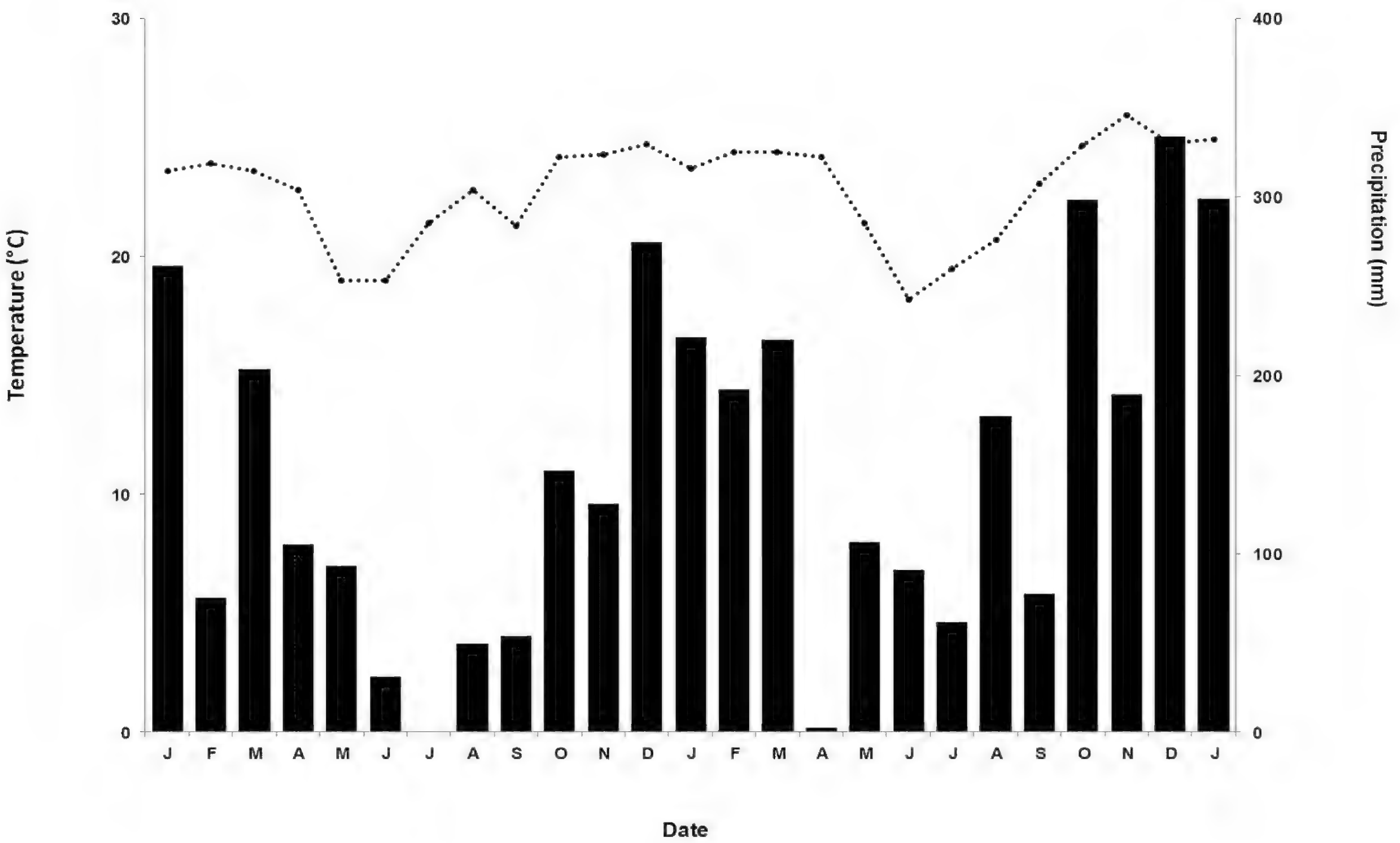

Figure 1. Climatic data from January 2008 to January 2010 for the municipality of Campo Grande, Mato Grosso do Sul, Brazil. The mean monthly temperature is represented by a dotted line; accumulated monthly precipitation is represented by bars (Source: Meteorological Station of Universidade para o Desenvolvimento do Estado e da Região do Pantanal).

P. carthagenensis occur exclusively in forest understory and preferentially in moister soils, near streams (see Faria et al., 2012 for details on plant distribution in the study sites). In the three sites, edaphic conditions are quite similar, with the soil classified as dark-red latosol (oxisol) (Theodoroviz & Theodoroviz, 2010). The climate is characterized by a pronounced dry season from May to September and a rainy season from October to April. Annual accumulated rainfall is approximately 1532 mm, and the mean annual temperature is 20° to 22°C (EMBRAPA-CNPGC, 1985). Data on monthly accumulated precipitation and mean monthly temperature for the entire study period were obtained from the Meteorological Station of the Universidade para o Desenvolvimento do Estado e da Região do Pantanal (UNIDERP) (Fig. 1).

FLOWERING PHENOLOGY AND NECTAR

Twenty *Psychotria carthagenensis* plants per floral morph in each population were randomly selected and marked for the analysis of flowering phenology. In these individuals the number of open flowers was recorded between January 2008 and January 2010. Monitoring was carried out on a monthly basis until flowering had been initiated and was then done on a fortnightly basis. The flowering pattern (duration and frequency of the phenophase) of the populations was classified according to Newstrom et al. (1994).

In November 2009, the peak of flowering season in the three studied populations, five flowers on each of 10 randomly selected plants of each morph in each population were selected randomly for measuring nectar volume and concentration. The flowers used to measure the nectar were bagged in the bud phase, with nectar measurement performed on the following day, between 1000 hr. and 1200 hr. Anthesis in *Psychotria carthagenensis* begins approximately between 0500 hr. and 0600 hr. and similarly in both morphs (Faria et al., 2012). Most focal observations were also performed in the morning such that the values reflect the availability of nectar to floral visitors, which allowed us to compare these values with the frequency of visits in this period. Volume was determined using a micro-syringe, and the sugar concentration was determined using a pocket refractometer (Dafni et al., 2005).

FLORAL VISITORS

Census of insect visitors was carried out in the morning (0700–1200 hr.; five censuses per population in 2008 and seven in 2009) and in the afternoon (1200 hr. and 1800 hr.; one census per population in 2008 and 2009). Most observations were made in the forenoon, because previous observations had indicated

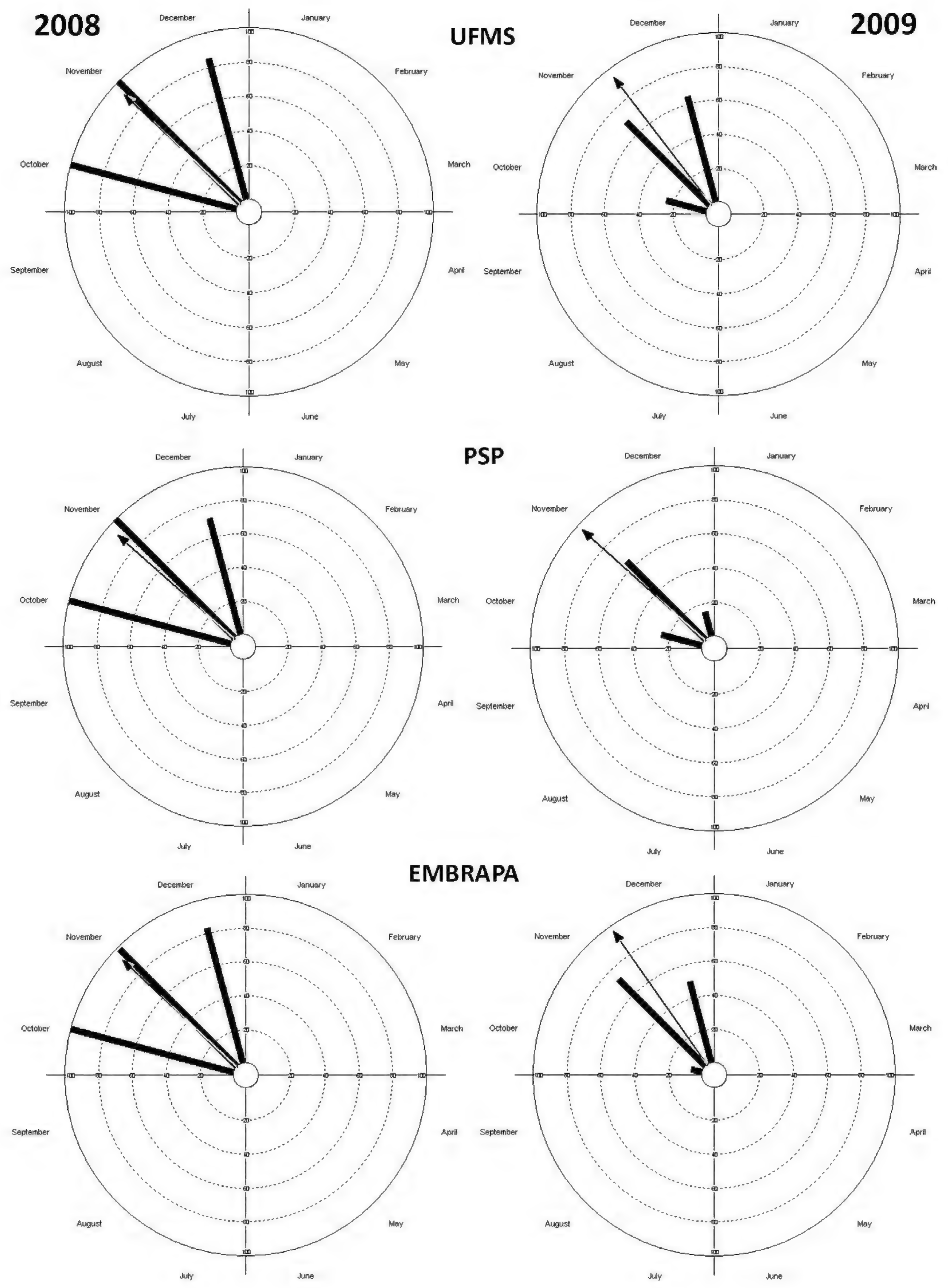

Figure 2. Circular histogram for flowering periods in three populations (UFMS, PSP, EMBRAPA) of *Psychotria carthagenensis* Jacq. in 2008 and 2009, Campo Grande, Mato Grosso do Sul, Brazil. Axes indicate frequency scale; bars indicate frequency of flowering individuals; arrow indicates mean flowering date; length indicates degree of synchrony between individuals within the population. The three studied populations are the Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

Table 1. Mean flowering date, synchrony (r indicates a concentration value in the mean angle; values range between 0 = absence of synchrony and 1 = complete synchrony), Rayleigh test and P values, with the level of data significance (< 0.05) for three populations of *Psychotria carthagenensis* Jacq. in 2008 and 2009, Campo Grande, Mato Grosso do Sul, Brazil. The Rayleigh test (z) calculates the probability (p) of the null hypothesis, which suggests that the data are evenly distributed, where z is the critical value to estimate this probability. The three studied populations are: Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA).

Year	Population	Mean date	Synchrony (r)	Rayleigh test (z)	P value
2008	UFMS	Nov. 3, 2008	0.924	243.242	< 0.001
	PSP	Nov. 3, 2008	0.924	232.113	< 0.001
	EMBRAPA	Nov. 1, 2008	0.931	243.614	< 0.001
2009	UFMS	Nov. 16, 2009	0.915	133.983	< 0.001
	PSP	Nov. 5, 2009	0.930	90.751	< 0.001
	EMBRAPA	Nov. 19, 2009	0.954	118.352	< 0.001

greater pollinator activity during this time (Rogério R. Faria, pers. obs.). The visitation rate (number of visitors/number of flowers observed/hour) was calculated through sessions of focal observations performed in the three populations. Each session was made up of focal observations of five randomly chosen plants of each morph. These observations lasted 15 minutes for each individual plant and were carried out every two weeks. Each observation session lasted 75 minutes per morph per population. Six censuses were performed in 2008 and eight were performed in 2009, totaling 360 hours of focal observations. According to a visitor's behavior, insect visits were classified as: (1) pollinating (contact with anthers and stigma) or (2) nectar thieving or robbing (no contact with anthers and stigma). Specimens of floral visitors were captured during the visits with the aid of a net and/or plastic bags for subsequent identification to the highest possible level of taxonomic resolution. Insect and plant vouchers are deposited at the Universidade Federal de Mato Grosso do Sul (Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul [ZUFMS] and Herbário da Fundação Universidade Federal de Mato Grosso do Sul [CGMS], respectively) (Appendix 1).

STATISTICAL ANALYSES

In order to determine the flowering pattern of the two floral morphs of *Psychotria carthagenensis*, circular histograms were constructed with the frequency distribution of flowering individuals in 2008 and 2009. For this analysis, days are converted to angles, from 0° (January) to 330° (December), in intervals of 30°. The Rayleigh test (z) calculates the probability (P) of the null hypothesis, which states that data are evenly distributed. The same test indicates the occurrence of periodicity in irregularly sampled data, in order to calculate the mean flowering date for each population and the concentration (r value, in this study considered as intra-population flowering synchrony) of blooming individuals around this mean date (Morellato et al., 1989), using the Oriana 2.0 program (Kovach, 2004). Thus, a P value below the level of significance (< 0.05) indicates that the data were not distributed evenly and offers evidence of a preferred direction. The length of the vector on the graph is related to the concentration value (r value, ranging from 0 to 1), and the arrow points to the mean angle (mean date) of the frequency distribution (Kovach, 2004). Floral display size was considered in each population as the maximum of flower production in each individual. Then, floral display size was compared between morphs in each population using a student t test. This test was also used to determine whether differences exist in the volume (μL) and concentration (%) of sugars in the nectar of pin and thrum flowers for each population separately.

Visitation rates for both morphs were calculated and compared separately by year and population using the non-parametric Mann-Whitney U test with the Bioestat 4.0 program (Ayres et al., 2005). The Morisita quantitative similarity index was used to calculate the similarity in the composition of floral visitors' species between morphs for each population and each year, with the aid of the Biodap program (Harmer et al., 2001). For this analysis, we calculated the visit frequency (number of visits of a species/total number of visits of all species per morph) of each floral visitor.

RESULTS

FLOWERING PHENOLOGY AND NECTAR

The flowering of *Psychotria carthagenensis* occurred between October and December in the three studied populations in both years (Fig. 2). Intra-population synchrony values (r) ranged from 0.915 to 0.954, and the mean date was between November 1 and 19. Significant values in the analyses were found in all cases (Table 1).

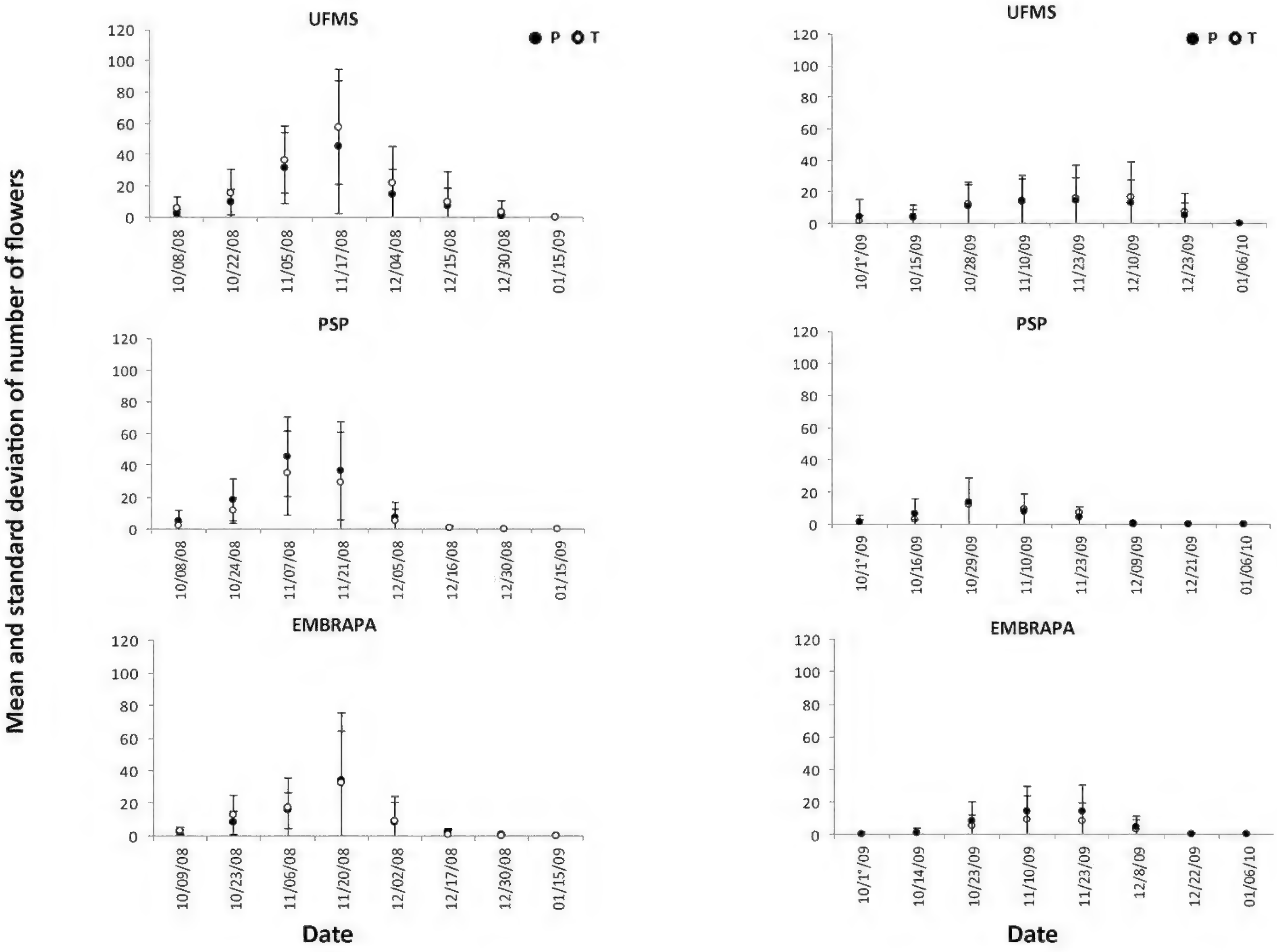

Figure 3. Mean and standard deviation of number of flowers of pin (P) and thrum (T) morphs in the three populations (UFMS, PSP, EMBRAPA) of *Psychotria carthagenensis* during flowering periods in 2008 and 2009, Campo Grande, Mato Grosso do Sul, Brazil. The three studied populations are the Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Closed circles represent mean values for P; open circles are mean values for T.

In 2008, peak flower production (mean and standard deviation) in the three populations ranged from 34 ± 41.32 flowers to 45.45 ± 24.88 flowers in the pin morph and from 32.05 ± 32.44 flowers to 57.6 ± 36.97 flowers in the thrum morph. In 2009, maximal flower production values in the three populations ranged from 13.74 ± 15.30 flowers to 14.6 ± 15.59 flowers in the pin morph and 8.85 ± 14.65 flowers to 16.1 ± 20.65 flowers in the thrum morph in 2009 (Fig. 3). The floral display size did not differ between morphs, both in 2008 and 2009, in all populations (Table 2). Also, means and standard deviations were never close to the peak of flower production, which is evidence that some plants reach their maximum floral display outside of the population flowering peak (Table 2).

Sugar concentration (mean and standard deviation) in the nectar ranged from 14.4 ± 5.96% in the thrum morph in the UFMS population to 16.6 ± 3.03% in the pin morph in the EMBRAPA population. Nectar volume ranged from 1.5 ± 0.68 μL in the UFMS population to 2.4 ± 0.52 μL in the PSP population. No statistically significant differences between morphs were detected for the concentration and volume data in any of the cases (Table 3).

FLORAL VISITORS

The highest visitation rates in 2008 were for the thrum morph in the UFMS population, for the thrum morph in the PSP population, and for the pin morph in the EMBRAPA population (Table 4). The highest visitation rates in 2009 were for the thrum morph in the UFMS population, for the thrum morph in the PSP population, and for the pin morph in the EMBRAPA population (Table 4). No significant differences were detected in the comparisons between morphs in each population (Table 4).

During the two flowering seasons (2008 and 2009), 22 floral visitors were recorded, with 14 visitors recorded in each population and only five common to all three populations. The Morisita-Horn index values

Table 2. Comparison of means and SD of floral display sizes (the maximum flower production in each individual) between pin (20 plants/population) and thrum (20 plants/population) floral morphs of three populations of *Psychotria carthagenensis* Jacq. from Campo Grande, Mato Grosso do Sul, Brazil. The three studied populations are the Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Student *t* test values are indicated by t; degrees of freedom are represented by df; *P* refers to values of significance.

	Population	Floral morph	Mean	SD	t	df	*P*
2008	EMBRAPA	pin	35.400	40.349	–0.149	35.847	0.883
		thrum	33.700	31.419			
	PSP	pin	53.250	12.384	0.919	26.961	0.366
		thrum	59.250	26.425			
	UFMS	pin	72.750	35.696	–1.276	24.046	0.214
		thrum	61.900	13.126			
2009	EMBRAPA	pin	17.550	16.168	–1.258	37.526	0.216
		thrum	11.450	14.442			
	PSP	pin	15.263	15.434	–0.277	36.903	0.783
		thrum	13.952	14.389			
	UFMS	pin	18.850	18.584	0.946	37.370	0.350
		thrum	24.800	21.135			

indicated a 99% similarity in the composition of visitors between the floral morphs in the UFMS population in 2008 and in the PSP and EMBRAPA populations in 2009. Ninety-one percent of similarity between morphs was recorded for the PSP population in 2008, 75% was recorded for the UFMS population in 2009, and the lowest value (21%) was recorded for the EMBRAPA population in 2008 (Table 5). The most frequent floral visitor in all populations was *Apis mellifera* L. (Apidae, honey bees), with a relative visiting frequency ranging from 1.8% to 67.6%, followed by *Augochloropsis* Cockerell (sp. indet., Halictidae, sweat bees), with a relative visiting frequency ranging from 1.8% to 17.6%. In particular, *Apis mellifera* showed some preference for thrum flowers, except in 2009 for the UFMS population (Table 5).

Among all the insects recorded in the flowers of *Psychotria carthagenensis* (Rubiaceae), only two were nectar thieves—ants and beetles. One beetle species (sp. indet., Curculionidae) and unidentified ants, including one species in *Camponotus* Mayr (Formicidae), visited flowers without coming into contact with the anthers in the thrum floral morph or the stigma in the pin morph. A weevil/snout beetle in the Curculionidae (sp. indet. 1) damaged the corolla near the flower's base, probably feeding on flower tissue, and did not contact the reproductive structures (cf. Table 5), so it acted as a nectar robber.

Table 3. Mean and SD of nectar volume (µL) and concentration (%) in pin (five flowers/10 plants/population) and thrum (five flowers/10 plants/population) morphs of three populations of *Psychotria carthagenensis* Jacq., from Campo Grande, Mato Grosso do Sul, Brazil. The three studied populations were the Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Student *t* test values are indicated by t; degrees of freedom are represented by df; *P* refers to values of significance.

Population	Nectar	Mean (± SD)	t	df	*P*
PSP	volume	pin, 2.1 µL (0.65) thrum, 2.4 µL (0.52)	–0.213	98	0.832
	concentration	pin, 15.5 µL (3.30) thrum, 14.6 µL (2.44)	–1.551	98	0.124
EMBRAPA	volume	pin 2.3 µL (0.50) thrum, 2.2 µL (0.44)	–1.124	98	0.264
	concentration	pin, 16.6 µL (3.03) thrum, 15.6 µL (3.39)	–1.492	98	0.139
UFMS	volume	pin, 1.5 µL (0.68) thrum, 1.6 µL (0.78)	1.059	98	0.292
	concentration	pin, 14.6 µL (5.87) thrum, 14.4 µL (5.96)	–0.169	98	0.866

Table 4. Range and median of visitation rates (number of visits/number of flowers/hour) between pin and thrum morphs of three populations of *Psychotria carthagenensis* Jacq. in 2008 and 2009, Campo Grande, Mato Grosso do Sul, Brazil. The three studied populations are the Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Mann-Whitney values are indicated by *U*; degrees of freedom are represented by df; *P* refers to values of significance.

Year	Population	Morph	Range	Median	*U*	df	*P*
2008	EMBRAPA	pin	0.10–0.38	0.25	12.000	1	0.34
		thrum	0.04–0.22	0.19			
	PSP	pin	0.07–0.25	0.20	19.000	1	0.87
		thrum	0.00–1.05	0.25			
	UFMS	pin	0.01–0.72	0.19	21.000	1	0.63
		thrum	0.03–0.78	0.16			
2009	EMBRAPA	pin	0.00–0.94	0.11	28.000	1	0.67
		thrum	0.00–0.88	0.06			
	PSP	pin	0.00–0.33	0.16	41.500	1	0.29
		thrum	0.00–0.57	0.14			
	UFMS	pin	0.00–0.17	0.00	31.000	1	0.92
		thrum	0.00–0.19	0.04			

DISCUSSION

Individuals of *Psychotria carthagenensis* were highly synchronous within the populations in relation to flowering, with an annual occurrence and mean flowering date occurring in mid-November. Populations with annual flowering and intermediate duration have only one main flowering episode in a 1-year period and a phenophase lasting one to five months (cf. Newstrom et al., 1994). Flowering phenology of species in the family Rubiaceae generally exhibits different patterns with regard to period, season, and flower abundance (e.g., Castro & Oliveira, 2002; San Martin-Gajardo & Morellato, 2003; Pereira et al., 2006), and there has been a consensus that phenology in this family does not undergo phylogenetic restrictions (Wright, 1991; San Martin-Gajardo & Morellato, 2003).

The beginning of the rainy season is the period of greatest abundance of insects in the Brazilian savanna (Pinheiro et al., 2002), and it is also normally associated with an increase in temperature and photoperiod, resulting in a phase of greatest reproductive and vegetative activity (Morellato, 1992; Morellato & Leitão-Filho, 1992). These characteristics indicate that both biotic and abiotic factors could exert selective pressure on the phenological pattern of *Psychotria carthagenensis*.

The synchrony presented by the *Psychotria carthagenensis* populations, with regard to both flowering season and intensity, favored greater intermorph floral visitation rates, as it allowed an equal probability of visitors for both floral morphs (in the case of isoplethic populations), an important factor to the stability of the heterostylous system (Ganders, 1979; Thompson & Dommée, 1993). Although important to the attraction of visitors and intermorph pollen flow, the similarity in floral display between floral morphs alone does not ensure similarity in reproduction success between morphs (Contreras & Ornelas, 1999). Actually, one of the major causes of morph-specific difference in reproductive success has been noted as the difference in the strength of heteromorphic incompatibility systems between floral morphs (Brys et al., 2008; Hodgins & Barrett, 2008). However, this difference was not detected here for *P. carthagenensis*, and, despite being isoplethic and presenting high morphological reciprocity between floral morphs, the studied populations are self-compatible and compatible within plants of the same morph (see Faria et al. [2012] for details on reproductive biology of *P. carthagenensis*).

The similarity in the reward offered to insect visitors by both floral morphs in *Psychotria carthagenensis* may have been responsible for the correspondence in the visiting patterns found in the populations. Indeed, no preference for one morph over another is expected when there is no difference in the volume and concentration of sugars (Weller, 1981; Wolfe & Barrett, 1987) or in the offer of flowers (Ganders, 1979) between floral morphs of distylous species. Exceptionally, *Apis mellifera* makes an apparent higher number of visits to thrum flowers. This preference by *A. mellifera* for thrum flowers, when both morphs present similar quantity and quality of nectar, as recorded in the studied populations, probably is due to spatial distribution of morphs and/or floral display in the neighborhood.

Table 5. Insect visitors and their relative frequency on flowers (number of visits of a given species/total number of visits of all species per morph) in pin and thrum morphs of three populations (Reserve of the Universidade Federal de Mato Grosso do Sul [UFMS], Prosa State Park [PSP], Reserve of the Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA]) of *Psychotria carthagenensis* Jacq. during flowering periods in 2008 and 2009, Campo Grande, Mato Grosso do Sul, Brazil. The similarity value (Morisita-Horn index) between morphs is presented for each population in a given year.

	2008						2009					
	UFMS		PSP		EMBRAPA		UFMS		PSP		EMBRAPA	
Visitors	Pin	Thrum	Pin	Thrum	Pin	Thrum	Pin	Thrum	Pin	Thrum	Pin	Thrum
Apidae												
Apis mellifera L.	29.2	47.3	17.3	67.6	1.8	15.2	32.8	23.2	33.5	49.1	36.7	39.2
Bombus L., sp. indet. 1								4.0				
Bombus, sp. indet. 2	1.9	3.0	0.4					3.2				
Bombus, sp. indet. 3					14.6							
Exomalopsis Spinola, sp. indet.						4.3					1.4	4.9
Lophopedia Michener & Moure, sp. indet. 1	0.4	0.4				1.2						
Lophopedia, sp. indet. 2			0.4		9.1	2.4						
Paratetrapedia Moure, sp. indet.	0.8	0.8	0.4	0.4	3.0	1.2		1.6		0.9	1.6	1.1
Paratrigona lineata Spinola			0.7		6.1	3.0			0.9	1.3	3.8	0.5
Tetrapedia Klug, sp. indet.					0.6	1.8			0.4	1.8	1.1	2.7
Xylocopa Latreille, sp. indet.							9.6					
Halicitidae												
Augochloropsis Cockerell, sp. indet.	5.7	3.4		1.8	14.6		1.6	17.6	5.8	3.1	2.7	2.2
Drosophilidae												
Drosophila Fallén, sp. indet.			0.4									
Nymphalidae												
Hypoleria lavinia Hewitson							3.2					
Lepidoptera												
Lepidoptera, sp. indet. 1		2.7	0.4	3.2				0.8	0.4			
Lepidoptera, sp. indet. 2					5.5	1.2						0.5
Vespidae												
Vespidae, sp. indet. 1	1.5		0.4									
Culicidae												
Culicidae, sp. indet. 1	0.4				1.8	2.4		1.6		1.8		0.3
Formicidae												
**Camponotus* Mayr, sp. indet.		1.1	0.4									
**Formicidae*, sp. indet. 1					0.6							
Curculionidae												
**Curculionidae*, sp. indet. 1		0.4	0.7		0.6							
NI		1.1	5.6	0.4	1.2	7.3		0.8		0.9	1.1	
Total number of visits	264		285		164		129		147		360	
Similarity (Morisita-Horn Index)	0.99		0.91		0.21		0.75		0.99		0.99	

* Nectar robbers.
Abbreviation: NI, not identified.

Given the floral morphology of *Psychotria carthagenensis*, contact with stigma by most of the recorded visitors was inevitable, so the majority of these insects acted as pollinators. Highly specialized flowers are rare in the family Rubiaceae, and the most common pollinators are generalist bees (Castro & Oliveira, 2002). For the genus *Psychotria* L., butterflies, hummingbirds, and wasps have been commonly recorded visitors (Almeida & Alves, 2000; Castro & Araujo, 2004; Teixeira & Machado, 2004). A generalist pollination system could be advantageous in situations of constant disturbance (Waser et al., 1996), as occurs in forest fragments situated in urban areas. Such fragments can serve as a refuge for anthophilous species and function as ecological islands, as favorable environments surrounded by an inadequate matrix (Cane, 2001)—civil construction in the present case. In general, the abundance of pollinators is reduced by the effect of fragmentation, especially in the reduction and isolation of the habitat (Kearns et al., 1998; Potts et al., 2010). However, the maintenance of a large number of visitors does not always signify reproductive success for plants (Gómez, 2002). In order to certify the generalization in this system, it would also be necessary to assess the effectiveness of the pollinators.

The EMBRAPA population had a low degree of similarity between floral morphs of *Psychotria carthagenensis* regarding insect visitors in 2008. This deviation may have occurred due to the quantitative nature of the similarity index used (Magurran, 1988). The two most frequent floral visitors (*Apis mellifera* and *Augochloropsis* spp.) to the thrum morph in the EMBRAPA population were not observed on the flowers of the long-style morph. Otherwise, the morphs shared eight of the 10 insect species recorded for the thrum morph and eight of the 12 species recorded for the pin morph. For distylous species, sharing the same pollinators and having similar visitation rates would allow for an increased likelihood of intermorph pollen flow (Husband & Barrett, 1992). Thus, specialization at the level of floral morph can be seen as detrimental, as leading to asymmetrical pollen flow and potential instability of the polymorphism (Wolfe & Barrett, 1987).

The similar flowering period, floral display, and visitation rates between morphs were indicative of successful intermorph pollen transfer in the studied populations of *Psychotria carthagenensis*, providing evidence for functional distyly in this species. However, further conclusions from these results are limited. Future studies designed for evaluating pollen flow rates between floral morphs are still necessary to better understand disassortative mating in *P. carthagenensis*.

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Appendix 1. Voucher numbers of collected specimens at the three studied populations: Prosa State Park (PSP), the Reserve of the Universidade Federal de Mato Grosso do Sul (UFMS), and the Reserve of the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA). Insects were deposited at Coleção Zoológica de Referência da Universidade Federal de Mato Grosso do Sul (ZUFMS) and plants in the Herbário da Fundação Universidade Federal de Mato Grosso do Sul (CGMS).

Specimens	Collector	Voucher number	Population
Psychotria carthagenensis Jacq.	R. R. Faria	52629, 52630	EMBRAPA
Psychotria carthagenensis	R. R. Faria	52633, 52634	PSP
Psychotria carthagenensis	R. R. Faria	52631, 52632	UFMS
Apidae not identified	R. R. Faria	HYM00285	EMBRAPA
Apidae not identified	R. R. Faria	HYM00270, HYM00272, HYM00275, HYM00276, HYM00277, HYM00278	UFMS
Augochloropsis Cockerell spp.	R. R. Faria	HYM00282, HYM00283, HYM00284	EMBRAPA
Augochloropsis spp.	R. R. Faria	HYM00287, HYM00290, HYM00291, HYM00292, HYM00295	PSP
Augochloropsis spp.	R. R. Faria	HYM00269, HYM00274, HYM00280	UFMS
Apis mellifera L.	R. R. Faria	HYM00281	EMBRAPA
Apis mellifera	R. R. Faria	HYM00286, HYM00288, HYM00289, HYM00293, HYM00294	PSP
Apis mellifera	R. R. Faria	HYM00268, HYM00271, HYM00273, HYM00279	UFMS